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GUIDELINES FOR HANDLING AND SHIPPING 'NEISSERIA MENINGITIDIS' F--ETC(U)  
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## SUFFIELD TECHNICAL NOTE

NO. 399

GUIDELINES FOR HANDLING AND SHIPPING *Neisseria meningitidis* FROM CF HOSPITALS TO DEFENCE RESEARCH ESTABLISHMENT SUFFIELD (DRES)

(REVISED) (U)

SUPERSEDES SUFFIELD TECHNICAL NOTE NO. 360

by

M.R. Spence and L.A. White



Technical Program 16 - Operational Medicine  
and

Tasks DPM 01 and DPM 19

JULY 1977

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ABSTRACT

Instructions are presented to ensure the transport of *Neisseria meningitidis* in viable state from remote sites to a diagnostic laboratory. Precautions are outlined which are necessary to prevent the accidental release of pathogenic material during shipment. In addition, instructions are provided for the handling of initial isolate material (e.g. on swabs or in cerebrospinal fluid (CSF)). The described shipping procedures are in accordance with IATA Packing Notes for Passenger and Cargo Aircraft, Nos. 701 and 720, Etiologic Agents. (U)

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INTRODUCTION

Under Task DPM 19, air samples are routinely collected in recruit barracks and the gymnasium at CFB Cornwallis and BFC St. Jean by base hospital personnel. These samples are then shipped to DRES where they are analyzed for the presence of *Neisseria meningitidis*. Samples from infected individuals as well as purified isolates from clinical cases are also sent to DRES for further investigation.

Special handling is required in order to maintain the viability of many bacterial pathogens during shipment. As well, when shipping pathogenic material, precautions must be employed to ensure that there is no possibility of release of this material into the transporting vehicle, thus causing a health hazard to crew or passengers. The onus is firmly placed on the shipper of such material to ensure that safety is not compromised. The International Air Transport Association (IATA) has recently published Packing Notes

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for Passenger and Cargo Aircraft with respect to etiologic agents (1). This report presents revised guidelines for the shipping of *N. meningitidis* which will replace those previously published (2).

Various media have been developed for the transport of bacterial pathogens while at the same time maintaining viability. Many are capable of allowing recovery of viable organisms for many days and, in some cases, even months. *Neisseriae*, however, tend to be very fragile and viability is not normally maintained for longer than 48 hours even in the better media (3) which are available. The time involved in transporting samples from CFB Cornwallis to DRES is usually greater than 48 hours. In fact, if the mails are employed (the usual means of transporting swabs of suspected pathogenic material), the delay may reach 10 days. Shipments have been received in which all samples were non-viable, whereas others in the same media and with a similar transit time have yielded positive isolates, thus indicating that temperature conditions also play a role.

An additional requirement which must be considered when transporting bacteria which will be employed in laboratory research on disease mechanisms is that the organisms arrive in the same morphological and physiological state as when they were shipped. For example, the presence of pili has been associated with virulence in *N. gonorrhoeae* (4,5) and recently DeVoe and Gilchrist (6) have demonstrated that on initial isolation from healthy carriers or from the cerebrospinal fluid (CSF) of disease cases, *N. meningitidis* are also heavily pilated. Pili are completely lost by third subculture on usual laboratory media. We have adopted a working hypothesis that, in this organism as in gonococcus, the presence of pili is related to virulence.

It has been shown that freezing to a temperature of -70°C will maintain viability of *N. meningitidis* for at least three months (Spence, M.R., F.W. Stevenson and L.A. White, unpublished data). Recent studies have found that the addition of dimethyl

sulfoxide (DMSO), at a level of 5-10%, to the freezing medium enhanced survival after freezing and thawing (7). Past experience has shown that Iso-Therm<sup>R</sup> (Canlab) containers will adequately maintain samples in a frozen state for at least four days if the samples are packed with good quality dry ice. Previous instructions have been included in earlier correspondence and signals, as well as in Suffield Technical Note No. 360, and it is the purpose of this note to provide complete and definitive guidelines for the packaging and shipping of *N. meningitidis*, which will comply with IATA regulations.

HANDLING OF SAMPLES

1. Samples collected from the air, broth cultures of isolates, and cerebrospinal fluid (CSF)

Samples collected 1) from the air by means of the DRES large volume air sampler (8,9,10), 2) from cerebrospinal taps and 3) trypticase soy broth (TSB) (Baltimore Biological Laboratories) cultures of *N. meningitidis* isolated from carriers, or disease cases, must be frozen immediately in collecting fluid containing 10% DMSO. Immediate and rapid freezing has been found to be essential since aerosolized meningococci rapidly die on standing in the collection fluid (7). It is recommended, therefore, that air samples be transferred immediately to a screw-capped tube and frozen on dry ice without delay. An Iso-Therm container filled with dry ice should be available at the sampling site for this purpose. The screw-capped tubes should be just large enough to contain the sample allowing for expansion due to freezing. In most cases, tubes approximately 100 mm x 13 mm are adequate. They should be filled to within 10 mm of the top if sufficient sample is available. It is essential that the top of the tube be sealed with a non-porous tape such as "gun tape"\*\* prior to freezing. This will prevent the entry of CO<sub>2</sub> into the tube. High concentrations of this gas may result in the death of *N. meningitidis*.

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\*Tape, pressure sensitive, adhesive, plastic coated, cloth backing, opaque, oil and water resistant, e.g. NATO No. 7510-21-116-9697 (TAN).

II. Samples collected on swabs

Nasopharyngeal swabs or other samples collected by fabric or surgical cotton swabs should be placed in screw-capped tubes containing 1.0 ml of TSB and 10% DMSO. The excess wooden handle must be removed and the screw cap sealed with non-porous tape. The sample must then be frozen immediately on dry ice.

III. Instructions for packaging of samples for aircraft shipment

Frozen samples are to be placed in an Iso-Therm container which will be provided by DRES. Fill the container to approximately one-third its volume with cracked (not crushed) dry ice. The primary containers (screw cap test tubes, sealed with a waterproof tape) must be wrapped in a continuous piece of cheesecloth, taking care to ensure that each tube is cushioned from the others. When so packed the tubes should be securely fastened by passing adhesive tape around them. The bundle must then be wrapped in a layer of absorbent cotton, placed in the small plastic bag supplied and sealed. Place these in the container and fill with more dry ice. Then seal the unit. Less than 5 pounds of dry ice is required for the packing as described above but it is recommended that it be obtained in 25 pound lots to ensure that sufficient quantity will be on hand to pack adequately the samples in case of delay. No more than 8 tubes should be placed in one bundle and no more than 2 bundles should be placed in a single Iso-Therm container. Total liquid volume per shipping container must not exceed 50 ml.

Place the container in a plastic bag and seal by means of a bag tie. Wrap it inabsorbent material (cheesecloth is suitable) which has been saturated with a 1:100 solution of Savlon\* in water. No other bactericide is acceptable. Savlon, at very low concentration, has been shown to be a highly effective disinfectant for this organism (11). Seal the unit in another plastic bag and place it in the plywood shipping box which is

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\*Savlon - Trademark; Ayerst, McKenna and Harrison.

supplied by DRES with the Iso-Therm container. This box must be securely fastened and addressed to its destination. The exterior of the box is to be clearly marked in the following manner:

F R A G I L E  
MEDICAL SUPPLIES AND BIOLOGICAL MATERIAL  
KEEP FROZEN  
PACKED IN ACCORDANCE WITH IATA REGULATIONS  
FOR SHIPMENT OF ETIOLOGIC AGENTS

SHIPMENT OF SAMPLES

Samples are to be shipped by air, preferably via Canadian Forces Flight No. 712\*. Shippers are required to contact the appropriate Air Movements Unit (for shipments from CFB Cornwallis - 3 AMU, Shearwater; for shipments from BFC St. Jean - 3 AMU, Ottawa) and arrange for the samples to be forwarded to 1 AMU, Namao on the earliest flight. The following authority must be quoted:

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Samples shipped to DRES via SF 712 to Namao, and then via Time Air to Medicine Hat, must be labelled in the following manner:

Chief/DRES  
CFB Suffield  
Ralston, Alberta

Attention: Preventive Medicine Section

The Preventive Medicine Section must be contacted by telephone by the shipper. CFB Suffield can be reached through CSN network. The waybill number and flight departure and arrival times must be reported. 1 AMU, Namao, will handle shipments in a manner previously set up (Letter, CFB Edmonton - DRES, 7505-1 (BTNO) dated 10 June 1975, signed by Major H.R. Christianson,

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\*Shipments must be designated as dangerous cargo because of the dry ice in which the samples are packed. The amount of dry ice used in any container is less than 5 lbs which is far below the Safe Dry Ice Loading listed in CFP 117, Article 1121, para 3(a). This fact should be brought to the attention of AMU personnel in order to avoid problems in the acceptance of shipments.

as amended by Letter, DRES-CFB Edmonton, DRES 9365-10-4 (Prev. Medicine) dated 25 June 1975, signed by Mr. J.F. Currie). If flights are delayed, or if the sample inadvertently is not placed on the appropriate flight, or if a delay is experienced in transshipping, the container must be held in a deep freeze during the delay.

SUMMARY

If the samples containing *N. meningitidis* are handled as described, they will reach their destination in a viable condition, essentially physiologically and morphologically unaltered, and at the same time will have presented no risk to those having handled them. In summary, the steps to be followed are re-emphasized:

1. Place samples in small tubes and freeze.
2. Seal tubes, wrap as described in Handling Samples, Section III, and seal in a small plastic bag.
3. Pack samples in dry ice in an Iso-Therm container.
4. Pack the container in such a way as to ensure organisms will be decontaminated in case of accident.
5. Place the container in the properly labelled shipping box.
6. Arrange with appropriate AMU detachment for shipment to DRES.
7. Contact DRES.

NOTE:

To facilitate the identification of samples, each shipment must include a note which designates the shipping date. Copies of the Government Bill of Lading (GBL) are to be placed in an envelope and firmly fixed to the container and not sent separate from it. In addition, one copy of the GBL must remain with the container until it reaches its final destination, DRES.

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Suffield Technical Note No. 374. Unclassified.

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2. *Neisseria meningitidis*
3. Transport media
4. Transport
5. Viability
6. Disinfection

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